Section A-Research paper
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FORMULATION AND EVALUATION OF TAMARINDUS INDICA BASED GEL FOR ITS IN-VIVO ANTIINFLAMMATORY ACTIVITY

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ABSTRACT

Aim: The development and evaluation of an herbal anti-inflammatory gel employing a hydroethanolic extract from Tamarindus indica seeds is the current goal of our study.

Methods: The gels were made with carbopol, various extract concentrations, propylene glycol, methylparaben, and the required volume of distilled water. Triethanolamine was gradually added to the gel, keeping the pH between 6.8 and 7.4. The produced compositions' physical characteristics, pH, spreadability, viscosity, and skin irritancy were evaluated. The anti-inflammatory activity was evaluated in albino Wistar rats of either sex (150-200 g) using a carrageenan-induced rat paw edema paradigm. The changed edema volume in the rat hind paw was calculated, along with the percentage of inhibition.

Results and Discussion: The findings showed that the gel had good viscosity, spreadability, homogeneity, and appearance. Any formulation tested on animals did not cause any skin pain. The skin preparation did not irritate the skin when applied to it, and neither did it create erythema or edema. In contrast to the usual medication, which reduced inflammation by 47.29% at 4 hours and 55.69% at 5 hours, Formulations F3 significantly reduced inflammation to a level of 48.64% at 4 hours and 53.16% at 5 hours, respectively. The anti-inflammatory effects of herbal gel (F3) showed significant difference ($p<0.05$) with negative control group. The anti-inflammatory effects of herbal gel (F3) and the common commercial diclofenac gel did not differ significantly ($p<0.05$). Herbal gels boosted the anti-inflammatory mediators' inhibitory actions. The hydroethanolic extract of Tamarindus indica seeds demonstrated the presence of flavonoids, saponins, and tannin components, suggesting the seeds' potential anti-inflammatory effect.

Conclusion: The anti-inflammatory effects of F3 were found to be equivalent to those of other standard drugs.

Key words: Herbal anti-inflammatory gel, *Tamarindus indica*, diclofenac gel, Carrageenan.

Introduction

Pain, redness, swelling, and malfunction of the tissues and organs are the basic manifestations of inflammation in humans. It is a typical result of the host's defense mechanisms against tissue damage brought on by a range of stimuli (such as physical trauma, poisons, and infectious agents) [1-2]. Inflammatory diseases, such as arthritis, heart disease, diabetes mellitus, and others, have grown to be significant public health issues in recent years, which has increased the annual mortality rates. As a result of the release of analgesic mediators, inflammation is typically followed by pain.[4] The presence of numerous hazardous chemicals triggers a variety of intricate biological reactions known as inflammation, which cause cellular and tissue damage. Its processes, which have a protective purpose, work to eliminate the root of cell harm and start the process of tissue healing.[5] The immune system produces a number of pro-inflammatory mediators, including interleukins, tumor necrosis factor alpha, reactive oxygen species, nitric oxide, and prostaglandins, which are released when tissue homeostasis is disturbed.[6-7] As a result, the local microcirculation is impacted, and through vasodilation and increased vascular permeability, other blood proteins and blood cells that mediate the inflammatory response are drawn to the location of the damaged tissue.[8] The main topical medications used to treat skin inflammation are corticosteroids and nonsteroidal anti-inflammatory medicines (NSAIDs). Inflammation is a defining feature of many chronic disorders.[9] However, using these medications frequently and for an extended period of time to treat inflammatory diseases can have unfavorable effects such as pruritus, irritations, dry skin, folliculitis, hypertrichosis, acneiform eruptions, hypopigmentation, allergic contact dermatitis, skin maceration, stretch marks, and military.[10-11]Research is therefore required to find anti-inflammatory and analgesic substances that have fewer side effects and greater efficacies. In addition, one of the most important sources for finding novel medicines with a large safety margin is herbal mixes.

The steroidal and non-steroidal anti-inflammatory medications that are readily available have a variety of negative effects. In order to find natural anti-inflammatory drugs, numerous studies are being conducted. Many plants are employed in conventional medicine. The bioactive phytochemicals found in abundance in the herbal materials used in preparation include polyphenols, carotenoids, flavonoids, lignans, phytosterols, and compounds found in essential oils with considerable analgesic and anti-inflammatory properties. [12-13]

Tamarindus indica seeds have reportedly been found to have anti-inflammatory, anti-asthmatic, anti-diabetic, and antioxidant properties. [14-15] Additionally, Tamarindus indica seeds are a good source of polymeric tannins, fatty acids, flavonoids, saponins, alkaloids, and glycosides. [16-17] Because they function as antioxidants and possible cyclooxygenase, lipoxygenase, and nitric oxide synthase inhibitors, flavonoids, tannins, saponins, and alkaloids are responsible for the anti-inflammatory and analgesic effects. [18,19,20]

Despite the fact that Tamarandus indica has been used to treat inflammation, there is no information on the creation of topical gel formulations using tamarandus indica seed hydroalcoholic extract. Therefore, the goal of the current study is to develop and evaluate an efficient topical gel anti-inflammatory formulation using tamarndus indica hydroalcoholic extract.[21] To bring tamarandus indica seed herbal gel for general therapeutic use, nonetheless, more in-depth research is required.

This will both demonstrate its efficacy in treating the intended illness and facilitate its adoption. Therefore, the goal of the current study was to evaluate the anti-inflammatory and analgesic effectiveness of tamarandus indica seed herbal gel in an experimental animal model after a single application and to compare this activity to that of the reference drug diclofenac.[22]

Material & Methods:

Drugs and reagents:

The seeds of T. Indica were purchased from the local market of Azamgarh, Uttar Pradesh. The plant was identified and authenticated by BHU, Varanasi U.P. Carrageenan was procured from Purvanchal Scientific Varanasi U.P. All other chemicals and reagents used were of analytical grade (Purvanchal Scientific Varanasi U.P.).

Experimental animals:

Adult Wistar albino rats of either sexes, weighing in about 180–200 g was procured. The animals were housed in cages at a temperature of 25°C with unrestricted access to water and a regular pellet meal. They were given a week to become used to the lab environment, and wherever necessary, they fasted the night before an experiment. The trials for a particular rat group were carried out in accordance with GLP (Good Laboratory Practice) standards. Before beginning the rat study, permission was obtained from the Institutional Animal Ethics Committee (IAEC), R.K. Pharmacy College, Azamgarh (U.P), India (Registration number**: 1384/PO/RE/S/10/CPCSEA).**

Preparation of hydroethanolic extract:

The Soxhlet process was used for extraction of the seed. As a solvent, a mixture of 99% ethanol and distilled water was utilized at a ratio of 1:5. Round bottom flask (RBF) was filled with 500 ml of hydroethanolic solution and RBF was then set on the heating mantle. The thimble was filled with 100 g of powdered plant material before being placed inside the Soxhlet extractor. The condenser was put on top of the Soxhlet extractor, which was positioned on top of the flask with a circular bottom. Running tap water was attached to the condenser's water intake, and water continued to flow through it before the heating mantle was turned on. at 30 to 40°C, the heated solvent started to evaporate and passed through the condenser. The liquid that had condensed was poured into the thimble. One cycle was finished when the solvent level reached the syphon tube and was then dumped into the round-bottom flask. The technique was repeated and continued for 12–14 hours. The extract was dried and concentrated. The dried extract was then ground up in a mortar and pestle and passed through sieve 40 to produce a fine powder.
The dried hydroethanolic extract was utilized throughout the trial and preserved in an airtight container in a desiccator.

Pre-formulation screening of extract:

Percentage yield of extract: The hydroethanolic extract yield was measured as a percentage.

Physical characterization of extract: Tamarindus indica seed extract was tested for its appearance, including colour, texture, and aroma

Determination of pH of extract: - Tamarindus indica seed extract's pH levels were assessed using a calibrated digital pH meter. Three duplicates of each measurement were made.

Phytochemical analyses of extract: By putting the extract through a qualitative examination and the phytochemical components were identified. According to accepted procedures, the hydroalcoholic extract of T. indica seeds underwent a preliminary phytochemical examination. [23,24]

Formulation of herbal gel:
Weighed methyl paraben (0.2 g) was added to warm water, mixed, and allowed to dissolve. After being weighed, the extract was diluted in 15 mL of propylene glycol. The extract mixture was added to the methyl paraben that had been dissolved. A clear and translucent gel was produced when 1 g of carbopol was measured, sieved, added to the liquid, made up to 100 mL, and swirled constantly for 30 minutes with a stirrer. Tamarindus indica seeds extract were utilized in varying amounts for each of the three formulations, as shown in Table 5.3. [25]
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Table 1. Composition of herbal gel formulations.

S. No.	Ingredients	F1	F2	F3	Blank Gel
1.	Methyl paraben	0.2	0.2	0.2	0.2
2.	tamarindus indica seeds extract	1.0	1.5	2.0	0.0
3.	Propylene glycol	15	15	15	15
4.	triethanolamine	qs	qs	qs	Qs
5.	Carbopol	1.0	1.0	1.0	1.0
6.	Water q.s.	100.0	100.0	100.0	100.0

Post formulation studies

Physical Evaluation

The gels' organoleptic characteristics, including odour, colour, and texture, were evaluated. Other physical tests, such as how simple it was to apply and remove were carried out.

Physiochemical Characterization of Formulation

Spreadability:-

With a few minor adjustments from Meera et al. (2010), 1 g of gel was poured on horizontal glass plates. The second glass slide was placed on top of the gel and time taken for spreading of gel was recorded. The diameter of the gel was also measured. This test was conducted three times for each formulation.

Grittiness: -

Under a light microscope, the formulations were examined for the existence of any particle debris. As a result, the gel preparation supports the need for any topical preparation to be free from any specific material or debris.

Measurement of pH

A calibrated digital pH meter was used to measure the herbal gels' pH values. Three readings of each measurement were made in triplicate.

Determination of viscosity: -

The Ostwald Viscometer was used to calculate the viscosity of herbal gel at room temperature. The tests were completed and documented in triplicate.

Skin irritation test of formulations

Wistar albino rats were lightly chloroformed anaesthetized before having their dorsal backs shaved to prevent tissue injury. The rat's dorsum was next cleaned with a 1% detergent solution, dried, and then a test area of around 3 cm^2 was enclosed. The animals' dorsum's were covered with filter sheets (2 cm^2) that had been treated with 100 mg of a specially made herbal gel and taped down with hypoallergenic tape. Samples were kept in contact with the skin of the animals for 4 hours before being removed using a 1% detergent solution. All animals were visually inspected at 1, 24, and 48 hours for indications of erythema, edema, dry skin, as well as any local or systemic clinical ill effects, including changes in behaviour, body weight, and food and drink intake.

Carrageenan-induced paw edema (acute inflammation)

Five groups of five animals each were assembled by randomly dividing the animals. To cause paw edema in the right hind paw of rats, a sub-plantar injection of 0.1ml of a 1% (w/v) suspension of -carrageenan was administered. Following the injection of carrageenan, the animal paws were exposed to five different treatment options: Group I as control, Group II, III and IV as test group and Group V as standard group. After 30 seconds, the rat received topically applied treatments of 30 mg of the placebo (placebo) gel, herbal gel, and 1%w/w diclofenac gel (anti-inflammatory positive control) in the paw where inflammation had been produced. A plethysmometer was used to measure the paw volume before the carrageenan injection and at intervals of 0.5 hours, 1 hour, 2 hours, 3 hours, 4 hours, and 5 hours afterward. Anti-inflammatory activity was determined by comparing the percentage of inhibition of the oedema substance to that of the control. Using the formula, the % inhibition of edema was determined;

Percentage inhibition of edema = (V_o-V_t) /To x 100

Where, V_t represents paw volume of rat given test extract at corresponding time and V_o is the paw volume of the rats of control group at the same time.

Statistic analysis

The findings of each in vitro experiment were done in triplicate and represented as mean ±S.D. The findings of the in vivo study's comparison of the ER and EI data between the treatment groups and the vehicle group were represented as mean ±S.E.M. Graph Pad Prism software was used to investigate the significant difference ($p<0.05$) using a one-way ANOVA.

Results:

Authentication of plant:

Prof. Nawal Kishor Dubey, Department of Botany, Banaras Hindu University Varanasi, India, approved the plant. For future reference, a voucher specimen no. Composita. 2023/01 was kept at the herbarium of BHU Varanasi.

Percentage yield of extract: Tamarindus indica seed's hydroethanolic extract was found to have a 10.7 percent yield.

Organoleptic properties of Extract -

Table 2. *T. indica* extract's organoleptic properties

Extract	Colour	Odour	Texture	pH
Hydroethanolic extract of tamarindus indica seeds	Light brown	Slight	Smooth	4.9±0.1

7.1.4. Qualitative phytochemical constituents of Hydroethanolic extract of Tamarindus indica seeds.

Table 3. Phytochemical constituents

S.No.	Phytochemicals	Test name	Observation	Result
1.	**Alkaloids**	**Mayer's test**	**Yellowish or white ppt.**	++
		Wagner's test	**Yellow or brown ppt.**	++
		Hager's test	**Yellow crystalline ppt.**	++
2.		**Lead acetate**	**Yellow colour**	++

	Flavonoids	**Alkaline reagent test** **Gelatin test**	**Yellow fluorescence** **White ppt.**	++ ++
3.	**Tannins**	**Ferric chloride**	**Blackish blue colour**	++
4.	**Saponin**	**Foam test**	**No foam formed**	--
5.	**Glycosides**	**Keller-killiani test**	**Reddish brown layer formed**	++
6.	**Protein**	**Biurate test**	**Pink or violet colour**	++
7.	**Amino acid**	**Ninhydrin test**	**Dark purple**	++
8.	**Carbohydrate**	**Benedict's test** **Molisch's test**	**No ppt.** **No ppt.**	-- --

Physical characterization

Table 4. Physical characterization of herbal gels.

Gels	Appearance	Colour	Odour	Texture	Ease of application	Ease of removal
F1	Less viscous	Light brown	Slight	Very smooth	Very easy	Very easy
F2	Thick, smooth	Deep brown	Slight	Smooth	Easy to apply	Easy to remove
F3	Thick, smooth	Deep brown	Herb like	Smooth	Easy to apply	Easy to remove

Table 5. pH, Spreadability and Viscosity of Herbal Gels.

Formulations	pH	Spreadability(mm)	Viscosity (pas)
F1	6.1±0.4	16.0±0.1	4.3±0.2
F2	5.9±0.5	18.0±0.6	4.5±0.4

F3	5.8±0.2	19.0±0.3	4.7±0.2

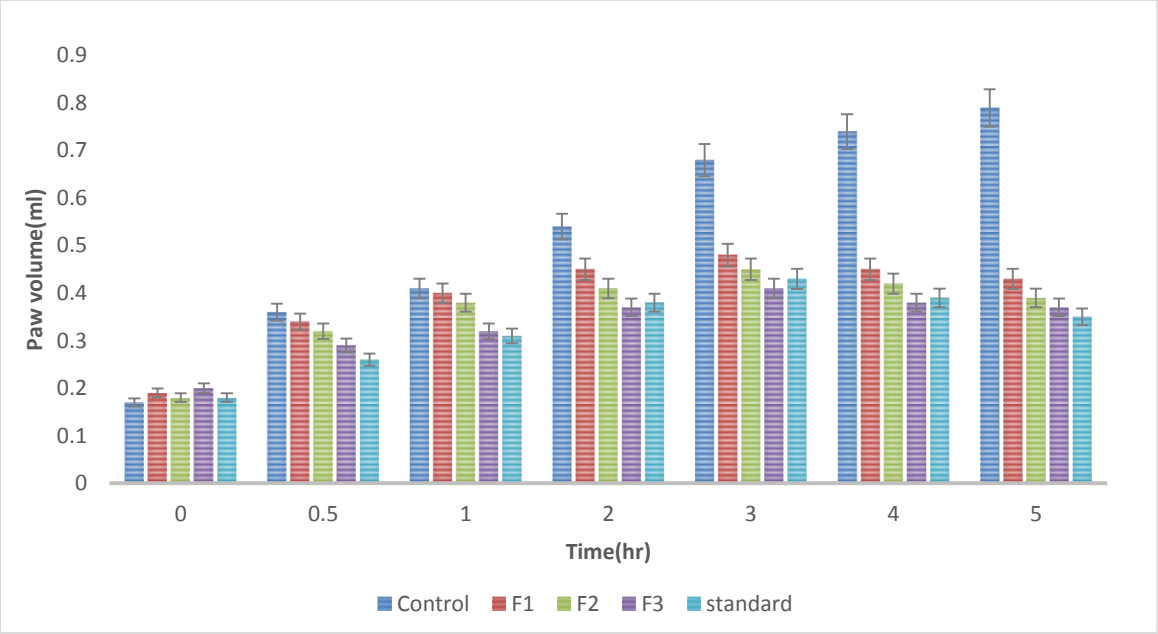

Fig 1. Topical anti-inflammatory effect of the different gels on carragenan induced rat paw edema.

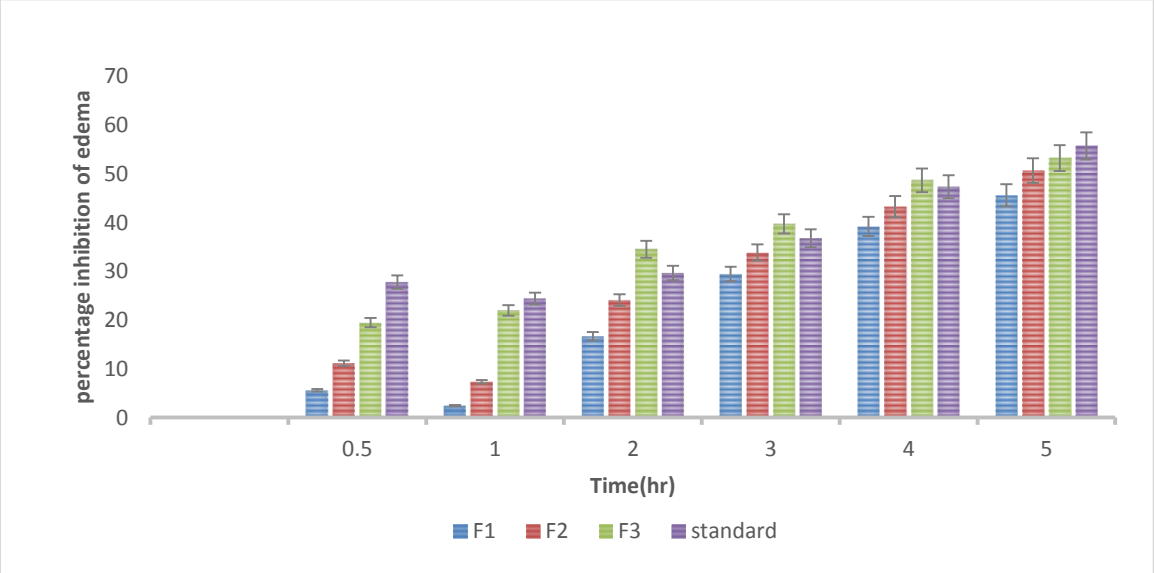

Fig 2. suppressive effect of hydroethanolic extract of tamarindus indica oncarrageenan induced hind paw edema in rats

Carrageenan induced rat paw edema result:

While the standard drug reduced the inflammation by 47.29% at 4 h and 55.69% at 5 h, Formulations F3 considerably reduced the inflammation to the amount of 48.64% at 4 h and 53.16% at 5 h, respectively. The hydroethanolic extract of the Tamarindus indica seeds demonstrated anti-inflammatory effect in the carrageenan-induced rat paw edema model. The inhibitory effects of mediators were strengthened by herbal gels. The anti-inflammatory effects of herbal gel (F3) and the common commercial diclofenac gel did not differ significantly ($p<0.05$).

Discussion:

The hydroethanolic extract of Tamarindus indica seeds' % yield indicated that there is not much more loss of usable raw material and that it is also reasonably priced. The extract had a tamarind seed-like color and odour. The smoothness of gel made it suitable for applying. The extract's pH was found to be slightly acidic. Tamarindus indica seeds were extracted to reveal their phytochemical composition, which included alkaloids, phenol, flavonoids, tannin, saponin, glycosides, proteins, and amino acids. According to the studies, flavonoids and polyphenols are what give our extract its anti-inflammatory effects. The gel's consistency was smooth and thick, making it suited for skin application. The gel's smooth texture and low odour make it simple to apply to topical areas. Herbal gel formulation had a pH level that was quite similar to skin pH. The gel was simple to apply on skin. Skin irritation test using a herbal gel formulation showed no any adverse effects.

The hydroethanolic extract of the Tamarindus indica seeds showed anti-inflammatory activity, according to the results of the carrageenan-induced rat paw edema model. The inhibitory effects of mediators were strengthened by herbal gels. Tamarindus indica seeds' hydroethanolic extract revealed the presence of flavonoids, saponins, and tannin components, possibly supporting its potential for anti-inflammatory activity. The major ways that these advantages were seen were by strengthening the body's antioxidant defenses and controlling inflammatory indicators.

Conclusion:

The key outcome of this study indicated how effectively a Tamarindus indica seed-based herbal gel made of hydroethanolic extract of inflammatory mediators could be used to treat inflammation. Herbal medications (herbal gel) may be superior to NSAIDs for long-term usage as a secure and efficient substitute for treating inflammation.

Future prospects:

Although Tamarindus indica has been used to treat inflammation, there is no information on the creation of a topical gel formulation using the hydroethanolic extract of the seeds. Therefore, the goal of the current study was to evaluate the anti-inflammatory effects of Tamarindus indica seed herbal gel in an experimental animal model and compare those effects to those of the reference medicine, diclofenac sodium.

To bring Tamarindus indica seed herbal gel for general therapeutic use, nonetheless, more in-depth research is required. This will both demonstrate its efficacy and make it more widely accepted as a method of treating inflammations.

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